The Journal of Parasitology

Volume 2 JUNE, 1916 Number 4

THE SIGNIFICANCE OF CERTAIN NATURAL FLAGELLATES OF INSECTS IN THE EVOLUTION OF DISEASE IN VERTEBRATES

H. B. FANTHAM

Lecturer in Parasitology, Liverpool School of Tropical Medicine, and Christ's College, Cambridge

AND

ANNIE PORTER

Beit Memorial Research Fellow, Quick Laboratory, Cambridge

INTRODUCTION

During the last few years, considerable attention has been given to the rôle of insects in the spread of disease. Much work has been done in elucidating life-histories, more particularly of the parasitic flagellates peculiar to insects and having no apparent connection with vertebrate maladies. Many flagellates that are seemingly limited to insects, however, are not so innocuous to vertebrates as they appear at first sight. The introduction of certain of the parasitic Mastigophora, notably members of the genera Herpetomonas and Crithidia, into vertebrates by the latter swallowing the infected insects or by forms of the parasite entering the vertebrate host by way of wounds or abrasions of the skin, has been shown to result in pathogenic effects to the said hosts. Laveran and Franchini have demonstrated the existence of this capacity for exercising latent pathogenicity by infecting dogs with the flagellates of dog fleas, and rats and mice with the flagellates of the fleas infesting these rodents. The present authors, working quite independently of Laveran and Franchini, have conducted a series of experiments extending over some six years on the possible pathogenic effects that accrue when certain flagellates of insects reach either associated or unassociated hosts. We have considered more especially the evolution of disease as exemplified by flagellates that have induced a flagellosis in vertebrates, remembering that, at any rate in some cases, parallel conditions prevail in Nature and in our experiments. By the introduction of certain herpetomonads normally parasitic in insects into vertebrates, a condition resembling leishmaniasis or kala-azar in man

has been produced, the symptoms of the disease and the morphology of the parasites found therein showing that here are, at least, examples of "leishmaniasis in the making."

The evolution of the parasitic habit with the development of pathogenicity as the result of change of habitat is no new phenomenon to those who have carefully studied the comparative morphology and life histories of various pathogenic Protozoa. Change of habitat has frequently led to great alteration in the mode of life of an organism. Thus, when a herpetomonad has so adapted itself that it is capable of living and propagating in a vertebrate, as has probably happened in the case of Leishmania, an originally monogenetic parasite by the exertion of its capacity for plasticity becomes digenetic. The various herpetomoniases, of which the leishmaniases are a special section, are probably the result of the introduction of insect or other invertebrate flagellates into the vertebrate hosts. In the case of acute forms of disease, the excitants show less perfect power of adaptation to the new environment than do the parasites that induce the chronic type of malady. From the point of view of the parasite, the maintenance of the life of the host is an economic desideratum, the prolongation of the active life of the invader depending in part on the longevity of the host. The newer a parasite is to the animal harboring it, the less it is in harmony with its environment. The consequence is that its discord with the host is manifested by pathogenic effects and the latter animal succumbs. Chronic maladies are usually correlated with greater powers of adaptation of the parasite to its host, with the period that has elapsed since the original introduction of the parasite to the host, and with the relative resisting powers of the host to the specific action of the parasite.

Certain trypanosomes appear to have developed from the flagellates of certain insects (for example Drosophilia), which parasites in turn seem to have been derived from free-living forms. As free-living organisms, their power for harm appears to be negative. If they become saprophytic, their capacity for developing noxious qualities is increased. When the parasitic habit of life, such as bloodsucking, is established in an insect, the power for injury possessed by its contained flagellates is greatly extended. Finally when the insect flagellate reaches the higher vertebrate the recapitulative effects of its evolution manifest themselves with cumulative results and the parasite is definitely pathogenic. The scale of evolution thus outlined is exhibited by the Trypanosomidae, some of whose members, the trypanosomes and herpetomonads (Leishmania) of vertebrates are notably pathogenic. While they are in the insect host, to which they have adapted themselves, they are relatively innocuous.

Yet other Protozoa afford studies in the evolution of pathogenicity. It must suffice to mention the case of the malarial parasites. The

morphology of these organisms shows that they are closely allied to the Coccidia, and there is little doubt that the malaria excitants were originally Coccidia of insects, that with change of habitat developed increased powers of adaptation to life in vertebrates, and, at the same time, increased in pathogenicity towards the new host.

The evolution of disease in the past is presented to us by the example of the malarial parasites. Disease in the making is manifested to mankind today in the case of the herpetomonads more especially. Preventive measures gain in efficacy as the natural modes of infection and possible sources of disease excitants are considered. In this connection, the experiments that we have undertaken may be of service as indicating possible sources of disease hitherto unsuspected.

MATERIAL AND METHODS

The materials used in this research consisted of various flagellates found in insects on the one hand, and of representatives of each of the great phyla of European vertebrates on the other.

The flagellates used in our experiments included members of the genera Herpetomonas and Crithidia. They comprised *Herpetomonas jaculum* Léger, parasitic in the gut of the Hemipteran, *Nepa cinera; H. stratiomyiae* Fanham and Porter, from the intestine of the Dipteran, *Stratiomyia chameleon; H. pediculi* Fanham, from the alimentary tract of *Pediculus vestimenti; H. culicis* Novy and MacNeal, from the larvae and adults of the gnat, *Culex pipiens;* and *Crithidia gerridis* Patton, parasitic normally in the alimentary tract of the Hemipteran, *Gerris paludum.*

The vertebrate hosts included representatives of the Pisces (*Gasterosteus aculeatus*), Amphibia (*Rana temporaria, Bufo vulgaris* and *Molge vulgaris*), Reptilia (*Lacerta vivipara* and *Tropidonotus natrix*), Aves (*Serinus canarius, Passer domesticus* and *Chelidon urbica*) and Mammalia (*Canis familiaris* and *Mus musculus*).

The insect flagellates were introduced into their respective vertebrate hosts either by inoculation or by feeding. In the latter case, the host was fed with the infected insects, or with the intestines of the insects, or with food contaminated with the feces of the insects containing the resistant (nonflagellate or postflagellate) stages of the Flagellata concerned. After the infective feed or feeds, ordinary food was given. The hosts were examined for blood parasites and for ectoparasites prior to use, and were found free from both classes of infestation.

Blood films were made periodically during the life of the infected animals, and smears of the internal organs were prepared at autopsy. Some preparations were fixed while moist with osmic vapor followed by absolute alcohol, while others were fixed wet with Bouin's fluid.

Intravitam staining was often employed. For permanent preparations Giemsa's stain, hematoxylin and eosin, iron hematoxylin and occasionally hematein were employed.

Control vertebrates were kept in each case. They remained healthy, lived longer than the experimental animals and were found to be unparasitized when killed.

EXPERIMENTAL WORK

The course of our experiments, extending over some years, on the introduction of insect flagellates into vertebrates may be gathered from the subjoined table. It has not been possible to manage a large number of animals satisfactorily at one time, as some have lived for relatively long periods. Sometimes it was possible to introduce the more resistant, encysted, leishmaniform, postflagellate forms of the Herpetomonas or Crithidia into the vertebrate. In such cases, it was found that a larger proportion of infections ensued than when the preflagellate or flagellate forms alone were introduced.

It is of some interest to note that as a rule the Herpetomonas or Crithidia introduced were few in number. The parasites, however, adapted themselves to their new surroundings, and both nonflagellate and flagellate organisms in all stages of active division have been recovered from the infected hosts. In some cases it has been possible to observe the completion of the multiplication in fresh preparations of the organs of the host. The parasites in the vertebrates are not merely conserved. The number of parasites obtained from the vertebrate host is superior to that introduced, multiplication of the organisms occurs and the host often undergoes pathogenic changes resembling those of leishmaniasis, that have frequently resulted in death. The herpetomoniasis induced is therefore regarded as a true parasitic infection, both the causal parasites and the maladies having affinities with kala-azar.

The possibility of bacterial contamination of the material used for feeding or inoculation has not been overlooked. Heavy bacterial contamination was exceptional in the insects used for experiment, and here, as elsewhere, the flagellates often die out in the presence of many bacteria. Further, in Nature pure cultures of flagellates or other protozoa rarely occur, as, for example, is evidenced by the diverse organisms found in sores of man in the East and in the mixed flora and fauna of the alimentary tract of vertebrates. Also, the insects swallowed by such vertebrates as lizards and snakes cannot, of necessity, contain pure cultures of flagellates or bacteria—in fact, mixed infections are frequent in Nature.

The tabular summary of our experiments is given on page 154.

From the following table, it will be seen that while a number of the infections are of the acute type, yet there are others in which the herpetomoniasis induced was of relatively long duration. We may mention that when the infection was of the chronic type, the leishmaniform, nonflagellate bodies preponderated in the smears of the organs of the vertebrate host, while flagellate herpetomonads were more numerous in the cases of acute herpetomoniasis. In practically all the animals infected experimentally, both nonflagellate and flagellate forms of the organism introduced were present, the proportion of each form showing variation. While these conditions prevailed in our experiments, we do not consider that any generalization can yet be made therefrom.

We would also point out that our experiments show the potential danger of many flagellates of insects that may at first sight seem unconnected with the vertebrates into which they have been introduced. Natural modes of infection, however, occur with a number of examples; thus, the dog may contract infection with herpetomonads by eating dog fleas and by ingesting infected flea feces when licking its coat, *H. jaculum* from *Nepa cinerea* can easily reach the fish and amphibia which it attacks and may even reach man by way of the wounds inflicted by the raptorial cutting forelimbs used when the insect sucks blood. The case of insectivorous birds whose normal food is insects is obvious. The experiments, whether between associated or unassociated insect flagellates and vertebrates, show "leishmaniasis in the making."

The chronic infections afford examples of good powers of adaptation to environment on the part of the parasites. As noted in our introduction, it is to the advantage of the newly established organism that the life of the host should be prolonged, and thus the continued existence of the parasite ensured. The acute cases are marked by the rapid development of the flagellate forms of the organisms, and by their less perfect adaptation to new surroundings as manifested in their pathogenic effects to their new hosts.

A further point of interest is that when young hosts were used, the parasites were more virulent. This is also the case with the parasites causing the human disease, Mediterranean kala-azar, which is prevalent more especially in children.

MORPHOLOGY OF THE PARASITES IN THE VERTEBRATE AND INVERTEBRATE HOSTS

The life-history of a herpetomonad in its insect host may be briefly outlined as follows: A *Herpetomonas* is a flagellate possessing also a nonflagellate stage in its life-cycle. This nonflagellate form is an ovoid or rounded, leishmania-like body containing a nucleus and a blepharoplast. It (Fig. 1a) may be passed from the host with the feces of the

TABLE—Results of the Experimental Infections of Different Vertebrates with Various Herpetomonas and Crithidia from Insects

No. of Experiment	Vertebrate Host	Flagellate Introduced	Mode of Introduction	Duration of Life of Host	Effect on Host	Forms of Parasites Observed in the Vertebrates	Remarks
1	Wild mouse, *Mus musculus*, ♀	*Herpetomonas jaculum*.....	Feeding.....	50 hours	Acute herpetomoniasis	Flagellate and nonflagellate	Young host
2	Wild mouse, ♂.....	*Herpetomonas jaculum*.....	Feeding.....	70 hours	Acute herpetomoniasis	Flagellate and nonflagellate	Young host
3	Wild mouse, ♀.....	*Herpetomonas jaculum*.....	Intraperitoneal inoculation	60 hours	Acute herpetomoniasis	Flagellate and nonflagellate	Young host
4	Wild mouse, ♂.....	*Herpetomonas jaculum*.....	Feeding.....	60 hours	Acute herpetomoniasis	Flagellate and nonflagellate	Host killed in extremis
5	Wild mouse, ♀.....	*Herpetomonas jaculum*.....	Feeding.....	84 hours	Acute herpetomoniasis	Flagellate and nonflagellate	Host killed when very ill
6	Wild mouse, ♂.....	*Herpetomonas jaculum*.....	Intraperitoneal inoculation	72 hours	Acute herpetomoniasis	Flagellate and nonflagellate	Host killed in extremis
7	Adult mouse, ♂.....	*Herpetomonas jaculum*.....	Intraperitoneal inoculation	Killed after 8 months	No symptoms of disease	Few non-flagellate..	Spontaneous cure, no parasites found at autopsy
8	Mouse, ♂.....	*Herpetomonas stratiomyiae*..	Feeding.....	5 days.....	Herpetomoniasis induced	Nonflagellate and some immature flagellate	Young host
9	Mouse, ♀, adult.....	*Herpetomonas pediculi*.....	Feeding.....	72 days.....	Herpetomoniasis induced	Mostly nonflagellate, very few flagellate forms	Chronic infection
10	Mouse, ♀, adult.....	*Herpetomonas pediculi*.....	Fed on liver of No. 9	15 days.....	Herpetomoniasis induced	Non flagellate and flagellate	.....
11	Mouse, ♀, adult.....	*Crithidia gerridis*.....	Intraperitoneal inoculation	40 days.....	Infection with *C. gerridis* induced	Flagellate and nonflagellate forms, the latter more numerous	Killed in extremis. Skin sore and alopecia at the site of inoculation
12	Mouse, ♀, adult.....	*Crithidia gerridis*.....	Subcutaneous inoculation	2 months, then killed	Negative	.....	.....
13	Mouse, ♂, adult.....	*Crithidia gerridis*.....	Feeding.....	38 days.....	Infection with *C. gerridis* induced	Flagellate and nonflagelate, the latter predominating	.....
14	Dog, *Canis familiaris*, ♂	*Herpetomonas ctenocephali*..	Feeding.....	Killed after 15 months	No marked permanent ill-effects	Nonflagellate	Young host. Spontaneous cure. No parasites found when killed

15	Canary, *Serinus canarius*, ♀, adult	*Herpetomonas jaculum*.....	Feeding.....	51 days.....	Chronic herpetomoniasis induced	Many nonflagellate, a few flagellate	Note chronic infection probably to be correlated with the presence of nonflagellate forms
16	Sparrow, *Passer domesticus*, ♀, adult	*Herpetomonas culicis*.....	Feeding.....	9 days.....	Acute herpetomoniasis induced	Flagellate and nonflagellate, the former predominating	Note acute infection, probably to be correlated with the presence of many flagellate forms
17	Martin, *Chelidon urbica*, ♂, young adult	*Herpetomonas culicis*.....	Feeding.....	12 days.....	Acute herpetomoniasis induced	Flagellate and nonflagellate, the former dominant	Note acute infection and many flagellate forms present
18	Martin, ♀, young adult	*Herpetomonas culicis*.....	Subcutaneous inoculation	2 days.....	.....	.....	Probably died of fright
19	Canary, ♂, young...	*Herpetomonas jaculum*.....	Feeding with infected insect excrement	17 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	.....
20	Martin, ♂, mature young	*Herpetomonas culicis*.....	Feeding with infected insect excrement	32 days.....	Herpetomoniasis induced	Nonflagellate and a few flagellate	.....
21	Sparrow, ♀	*Herpetomonas jaculum*.....	Feeding with infected insect excrement	Killed after 3 months	.....	One parasite only seen during life	Spontaneous cure. No parasites found at autopsy
22	Canary, ♀, adult....	*Herpetomonas culicis*.....	Fed on food contaminated with *H. culicis*	Killed after 80 days	Negative	.....	.....
23	Grass snake, *Tropidonotus natrix*, ♂	*Herpetomonas jaculum*.....	Feeding.....	20 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	.....
24	Lizard, *Lacerta vivipara*, ♂	*Crithidia gerridis*.....	Feeding.....	19 days.....	Infection with *C. gerridis* induced	Flagellate and nonflagellate	.....
25	Lizard, ♂	*Crithidia gerridis*.....	Fed on infected liver of No. 24	6 days.....	Acute disease (crithidiasis)	Flagellate and nonflagellate	Second passage
26	Lizard, ♀	*Crithidia gerridis*.....	Intraperitoneal inoculation with infected heart blood of No. 25	Killed after 20 days	Slight infection	Nonflagellate	Killed for examination after 20 days. Third passage
27	Frog, *Rana temporaria*, ♂, adult	*Crithidia gerridis*.....	Intraperitoneal inoculation	29 days.....	Infection with *C. gerridis* induced	Flagellate and nonflagellate	.....
28	Frog, ♂, adult.....	*Herpetomonas jaculum*.....	Intraperitoneal inoculation	54 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	.....
29	Toad, *Bufo vulgaris*, ♂, adult	*Herpetomonas jaculum*.....	Subcutaneous inoculation	40 days.....	Chronic infection ..	Nonflagellate and young flagellate	.....
30	Toad, ♀, adult.....	*Herpetomonas jaculum*.....	Intraperitoneal inoculation	80 days.....	Negative	None	.....
31	Newt, *Molge vulgaris*, ♂, young adult	*Herpetomonas jaculum*.....	Feeding.....	9 days.....	Apparently negative	None	Death by misadventure
32	Stickleback, *Gasterosteus aculeatus*, ♂	*Herpetomonas jaculum*.....	Feeding.....	2 days.....	.....	None	.....
33	Stickleback, ♂	*Herpetomonas jaculum*.....	Subcutaneous inoculation	6 days.....	Herpetomoniasis induced	Flagellate and nonflagellate	.....

latter, and is then surrounded with an outer coat. If the excrement containing the nonflagellate—sometimes termed encysted or postflagellate—forms of the herpetomonad is ingested by another insect host, these ovoid forms of the parasite have their firm, varnish-like outer coat (Fig. 1f) dissolved by the digestive juices of the host and are then capable of further development. In this condition, they are often termed preflagellate forms (Fig. 1a). The preflagellate form gradually elongates. A flagellum arises near the blepharoplast (Fig. 1c), reaches the surface of the body at the anterior end and finally projects as a free flagellum. The posterior end also elongates and thus the typical flagellate is produced (Fig. 1d).

Multiplication of the flagellate by longitudinal division can occur in either the nonflagellate (Fig. 1b) or the flagellate stage (Fig. 1e). As the organisms pass onward into the less favorable environment of

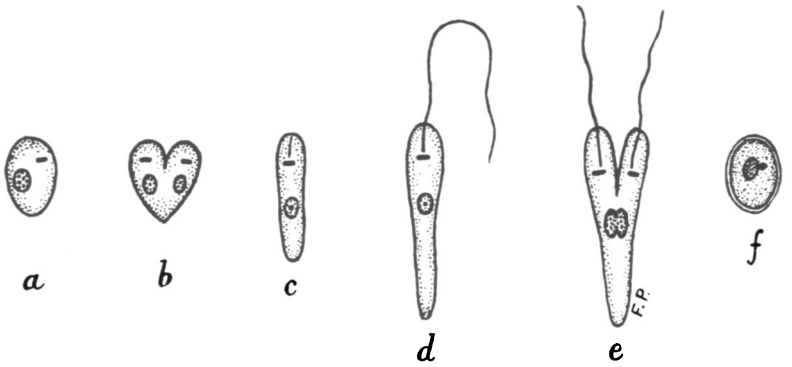

Fig. 1.—*Herpetomonas:* (a) non-flagellate or leishmaniform stage; (b) dividing non-flagellate; (c) elongating parasite; (d) flagellate stage; (e) dividing flagellate; (f) post-flagellate or encysted stage. × 1500.

the posterior end of the intestine of their host, their body cytoplasm concentrates, and the flagellum is withdrawn and largely dissolved. The now ovoid parasite secretes a coat which may be at first gelatinous but ultimately becomes varnish-like or "skin tight" and the postflagellate form is again produced. This resistant nonflagellate form (Fig. 1f) is particularly adapted for extracorporeal life and serves for the safe transference of the parasite from host to host.

The above outline of the life history of a herpetomonad is valid for *Herpetomonas jaculum, H. stratiomyiae, H. pediculi, H. culicis* and *H. ctenocephali* with which we experimented.

The life-history of a true *Crithidia,* such as *C. gerridis,* in its insect host has the same general outline as that of a Herpetomonas. But the flagellate stage differs from that of a Herpetomonas in that at the differentiation of a flagellum, this structure not only reaches the sur-

face, but forces the ectoplasm before it, thus producing a small wavy undulating membrane that gradually fades into the free flagellum at the tapering anterior, flagellar end of the body of the organism (Fig. 2b).

The morphology of *Herpetomonas jaculum, H. stratiomyiae, H. pediculi, H. culicis* and *H. ctenocephali* in the vertebrate hosts into which they were introduced resembled that in the invertebrate hosts. The parasites have been introduced both as flagellates and as nonflagellates. In blood smears taken during the life of the host and in organ smears made at autopsy, usually both flagellate and nonflagellate forms were found. Parasites in various stages of multiplication were

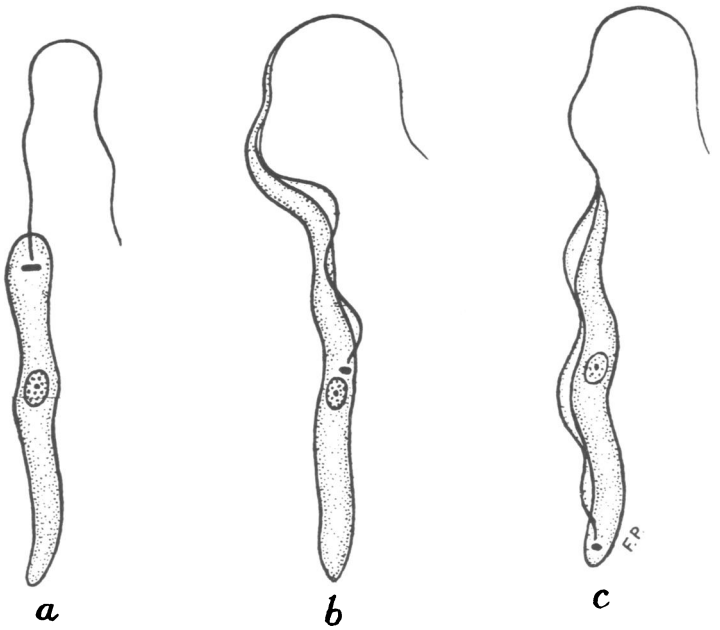

Fig. 2.—Flagellate forms of (a) *Herpetomonas* (sometimes called *Leptomonas*), (b) *Crithidia* and (c) *Trypanosoma*. × 2000.

observed in the fresh condition and in stained preparations. Hence there is definite evidence that they had become true parasites of the vertebrates, had established themselves and had increased in numbers in them, and were not mere conservations of the forms introduced.

The various Herpetomonas (Fig. 2a) and Crithidia (Fig. 2b) that we have used have retained the facies that they presented in the insect hosts. No transition to a trypanosome (Fig. 2c) was ever seen by us during the course of these experiments. The only variation presented by the parasites in the vertebrates from that in the invertebrates was that the maximum length of the insectan flagellate stage was not usually quite attained. The sizes of the parasites, however, were

always well within the range of the limits of variation given for the insect parasites and were of good average size. Morphologically, they were replicas of the insect forms and could be unmistakably identified with them. The nonflagellate forms were about the size of or slightly greater than the Leishman-Donovan body in man, while the dimensions of the flagellate forms were much the same as those of Leishmania in cultures on the Novy-MacNeal-Nicolle medium, that is, about 15μ to 20μ in the long diameter of body. The slightly lesser dimensions of the parasites may be the results of transference and implanting of the organisms in new hosts, or perhaps the age of the host may influence the size of the parasite. It has been noticed that other parasites, for instance, certain Haemosporidia, introduced into unfamiliar vertebrates or into young hosts tend to produce new generations whose maximum dimensions are somewhat less than those of their progenitors. The same factors may apply in this induced herpetomoniasis. On the other hand, it is known that the nonflagellate parasite of Indian kala-azar maintained in dogs may increase in longest diameter from about 2.5μ or 3.5μ to 8μ or 9μ. Similar variations in size occur in the nonflagellate stages of closely allied herpetomonads in insects.

THE SIGNIFICANCE OF CERTAIN NATURAL FLAGELLATES OF INSECTS IN THE EVOLUTION OF DISEASE

The rôle of insects in the spread of disease among men and other animals has furnished some of the most important advances in knowledge made in recent times. Many parasitic protozoa are the descendants of free-living ancestors. The degrees of degradation from independent life to saprophytism and thence to parasitism are almost imperceptible but nevertheless exist. Neither are the grades of parasitism more well defined, and consequently a free-living organism that by accident or chance has reached the alimentary tract of an insect may live there first as a saprophyte, feeding on the waste materials or the newly ingested food of the host. The minute quantities of nourishment lost by the host in this way become serious when cumulative, and the saprophytism then leads to parasitism of a somewhat low degree. When the living protoplasm of the host furnishes the nutriment required, the parasitism becomes obvious and the effects on the host are more or less marked.

In the case of many intestinal flagellates of insects, the host has responded to the attacks of the parasites in such a way that a mutual toleration has become established between them. Under these circumstances but little injury ensues to the host, and the flagellates concerned are considered as "natural" and practically harmless to the host. Further, they have often been considered as specific to the said hosts to which they are practically harmless.

Should such flagellates reach a vertebrate host, two courses may result. In the first instance, the flagellates may merely perish. In the second case, should the introduced organism be sufficiently plastic, it may adapt itself to its new environment and be able to persist for a time. Should its powers of adaptation be marked, it will multiply, and the greater the rapidity of increase, the greater the danger to the host. In other words, environment and plasticity determine pathogenicity.

Certain of the flagellates show the transformation from almost harmlessness in the insect to pathogenicity in the vertebrate or newer host. The genus Herpetomonas affords a good example of the capacity for pathogenicity that may be latent in many organisms hitherto considered harmless.

Kala-azar, oriental sore and dermomucosal leishmaniasis are well-known tropical diseases due to members of the Herpetomonadidae that are known as *Leishmania donovani,* and *L. infantum* in the cases of kala-azar, and as *L. tropica* in the more local maladies of the skin. These organisms are, in all probability, herpetomonads of insects that have reached vertebrate hosts. It is known that the various species of Leishmania develop into typical herpetomonad flagellates in cultures, and for some time now these flagellate stages have been known in man. Thus, in 1911, Escomel saw flagellate forms of *Leishmania tropica* in man and published about them later. La Cava in 1912 described similar forms of the same parasite in Italy. Also in 1912, Splendore found elongating forms and a few flagellate parasites in dermomucosal leishmaniasis in Brazil, while Monge in 1914, when working on the same malady in Peru, found the herpetomonad stage of the parasite. Lately (September, 1915) Wenyon has found the flagellate stage of *Leishmania donovani* in a dog subinoculated from other dogs, the strain being originally derived from a man who died of kala-azar contracted in Calcutta. Further, a new herpetomonad, *Haemocystozoon brasiliense,* was found by Franchini in 1913 in a human subject.

As a result of experimental work, such as that of Patton and of Wenyon, it has been shown that species of Leishmania can develop into herpetomonad flagellate stages within the intestines of certain insects, such as bedbugs and mosquitos (Stegomyia). The evidence that an ovoid Leishmania is the non-flagellate stage of a herpetomonas is proved, and the flagellate stage of Leishmania can exist in cultures, in insects and in man. Leishmania morphologically is a herpetomonad.

Herpetomonads experimentally introduced into vertebrates by us have produced pathogenic effects recalling those of kala-azar. Both maladies present the same features—the insidious onset, the subsequent relatively rapid illness, the splenic and often hepatic enlargement, feverish attacks and emaciation. In the cases where chronic infections

were produced in our animals, the nonflagellate, leishmaniform stages of the parasites were more numerous, while in acute cases the flagellate forms were more obvious (see table). In the diseases due to Leishmania spp. the flagellate forms in the vertebrate host are far less common than the nonflagellate ones, but it is of distinct interest to note that Monge (1914) suggested that the presence of flagellate forms of *L. tropica* in man was an indication of increased virulence on the part of the parasite. Such an increased virulence certainly coincided with more marked development of flagellates in our animals. Though no general conclusion on the subject can yet be given, the hypothesis that the presence of flagellate Herpetomonas or Leishmania in the vertebrate host affords an index of virulence is supported by the experimental results that we have obtained.

The part played by vertebrates proved capable of harboring herpetomonads is one that demands the attention of all students of preventive medicine and of sanitary reform. By experiment we have proved that flagellates belonging to the genera Herpetomonas and Crithidia have produced infections not only in mammalia like mice and dogs, but also in birds and in cold-blooded vertebrates such as members of the pisces, amphibia and reptilia. Further, these flagellates are capable of assuming resting, nonflagellate stages in these hosts.

There is thus the possibility that various vertebrates—fish, amphibia, birds, reptilia, and mammals—may serve as reservoirs of the herpetomoniases, including leishmaniases. The virus may be very attenuated and so escape detection, or only be revealed by the presence of flagellate forms in cultures. Recently (1914) Sergent, Lemaire and Senevet in Algeria have demonstrated the presence of a herpetomonad flagellate in the blood and organs of geckos obtained from areas in Algeria in which oriental sore due to *L. tropica* is present. Phlebotomus flies, which may harbor a natural herpetomonad, feed on the geckos and on man. Hence animals like geckos may possibly act as reservoirs of leishmaniasis. Chatton and Blanc (1914) have found possible leishmaniform bodies in the young red blood cells of geckos in Tunis. Bayon (1915) has found herpetomonad parasites in the cloaca of *Chameleon pumilus* at Robben Island, South Africa, and says that "it does not seem excluded that a chameleon can get infected through swallowing a fly containing Herpetomonidae in its gut." He also found a herpetomonad in the gut of the fly, *Scatophaga hottentota,* in the same place. Lindsay (1914) stated that the parasite of dermomucosal leishmaniasis in Paraguay is believed by native sufferers to be conserved in rattlesnakes and to be spread by ticks or flies (Simulium) feeding on the reptiles and transferring the parasite to man. We have shown the possibility of such infection occurring by causing insectivorous vertebrates, such as viviparous lizards and grass snakes, to

ingest insects infected with herpetomonads, wherewith the vertebrates became parasitised. Similarly, insectivorous birds have become parasitised by ingesting insects containing herpetomonads. These infections could be accomplished in Nature and, in fact, such parasitism of a bird by herpetomonads and of mice by the same flagellates has been found (see below). Natural reservoirs of herpetomoniasis, consisting of vertebrates on which sanguivorous insects feed, should be sought for in areas where diseases such as kala-azar are present.

Natural reservoirs of herpetomoniasis are already known. Man and his intimate domestic associate the dog, both may function as reservoirs of what has been termed Mediterranean or infantile kala-azar. The parasite, *Leishmania infantum,* which is often considered to be a form of *L. donovani,* is thought to be transmitted from dog to dog by the dog flea and possibly also from dog to man. An infected child or an infected dog may, perhaps, serve as the reservoir of the virus. In this connection it is of some interest to recall that cattle which have become immune to piroplasmosis may yet harbor sufficient sparse piroplasms in their blood to infect many ticks and so spread the malady. Analogy is somewhat dangerous, but in this case, it may be of service, since rare cases of "spontaneous cure" of infantile leishmaniasis are known and it is just possible that such may act as unsuspected reservoirs of leishmaniasis.

Vertebrates other than man can be infected naturally with herpetomonads. In 1903, Dutton and Todd described herpetomonads from the blood of house mice in Senegambia. The original description was very definitely that of a Herpetomonas, though Todd has recently stated that he thinks the organism may have been a trypanosome. However, we have also found herpetomonads closely resembling those described by Dutton and Todd in mice in England. It is known that the common rat-fleas contain herpetomonads and it is suggested that these fleas were the probable source of infection. Mice as possible reservoirs of leishmaniasis cannot be disregarded.

Again, a natural infection of birds has been described by Drs. Edmond and Etienne Sergent. In this case a pigeon was found to contain herpetomonads in its blood. The source of the flagellate is not known with certainty, but we advance the hypothesis that it was a latent herpetomoniasis contracted from herpetomonad-infected insects such as species of Lynchia that had fed on the bird.

From a careful comparison of natural and induced herpetomoniasis in vertebrates and of leishmaniasis, as well as consideration of the morphology and life phenomena of the excitants in each case, the following general statements can be made. Under suitable conditions, insect flagellates can be introduced into vertebrate hosts and can produce infections therein. In some cases, as in some cold-blooded verte-

brates, little obvious ill effect results; in others, as in mammals and birds, disease is manifested and often ends in death.

The organisms, such as herpetomonads, thus introduced, retain their powers of development on the same lines as when they were present in the insects. The morphological cycle is that of Herpetomonas. The various species of Leishmania are probably insect flagellates long since introduced into man and usually perpetuating the nonflagellate form, though capable of assuming the flagellate, herpetomonad facies in the internal organs of the vertebrate or in the invertebrate hosts.

No insect flagellate can be considered to be quite innocuous to vertebrates until it has been put to the test.

It must be remembered that leishmaniasis, which is a form of herpetomoniasis, is a flagellosis, as is also trypanosomiasis. The treatment of leishmaniasis by intravenous injection of tartar emetic—as advocated and practiced recently—is sound biologically, for drugs containing arsenic or antimony have proved efficacious in trypanosomiasis.

It is necessary to consider not part, but the whole, of the life history of an organism and also the relationship of the parasite to the group to which it belongs. There is a line of evolution common to each group and in these cases, neither Herpetomonas (Leptomonas), Leishmania, Crithidia nor Trypanosoma (Fig. 2) should be considered as isolated units but as flagellates belonging to the Trypanosomidae.

MODES OF INFECTION AND PREVENTIVE MEASURES AGAINST ARTHROPOD-BORNE HERPETOMONIASES

The experiments on the introduction of various species of Herpetomonas and Crithidia parasitic in insects into both warm and cold-blooded susceptible vertebrates has shown that these flagellates can produce an infection in the vertebrates when the latter are fed or inoculated with them. Within the host, the parasite is capable of assuming the leishmaniform or flagellate facies. The mode of infection of the vertebrate in nature seems to be contaminative, either by its food, or through an already existing abrasion or puncture on the surface of its body. The feces of insects, if containing the resistant forms of the flagellate, are capable of producing infection by similar channels. We have also obtained evidence showing that postflagellate forms of the parasite are the best adapted to begin life in a new vertebrate host.

Experiments on ourselves with fleas and lice, and with biting insects on rats, suggest that infection with Herpetomonas or Leishmania is not by inoculation with the protozoal parasites during the time when the insect is biting man or other vertebrate, but by the vertebrate eating the infected insect, or by infected insect feces passing through an abrasion, puncture or bite on the vertebrate skin. In this connection it is of

interest to note that Laveran has quite recently succeeded in infecting a mouse with a culture of *Leishmania tropica* by way of the mouth.

As we have already stated, in areas where leishmaniases are endemic, an examination should be made of all insects and other invertebrates likely to come into contact with men or dogs or rats and mice, in order to ascertain if these invertebrates habor herpetomonads. Preventive measures should be directed against such invertebrates, especially arthropods. Further, it is likely that certain vertebrates, such as reptiles and amphibia (especially such as are insectivorous), may serve as reservoirs for leishmaniases or, as they should preferably be termed, herpetomoniases. From such reservoirs the herpetomonads may reach man by the agency of ectoparasites or flies, especially such as are sanguivorous.

That some of these suggestions are of practical application has been proved by the work of Dodds Price in the Assam tea gardens, following on a suggestion from Rogers to the effect that action should be taken against suspected transmitters of kala-azar, even if complete inculpation of them had not been afforded. Dodds Price has reduced the mortality due to kala-azar enormously by segregating the infected, by moving coolie lines about three hundred yards from older, infected ones and by having new coolie lines placed on clean sites. Young (1914) has applied successful segregation measures to an indigenous population in certain villages in Assam. These measures check the prevalence of sanguivorous insects that infest man and his dwellings, and reduce the danger of possible infection by way of contaminated food or drink. It may be expected that the application of similar measures in other areas where kala-azar is endemic may also be equally efficacious.

SUMMARY

1. Herpetomoniasis can be induced in various warm and cold-blooded vertebrates when the latter are inoculated or fed with herpetomonads occurring in the digestive tracts of various insects. The infection produced and the protozoal parasites found in the vertebrates resemble those of human and canine leishmaniases.

2. An infection can also be induced in certain vertebrates when they are fed or inoculated with *Crithidia gerridis,* and both flagellate and nonflagellate stages occur therein, but no transition to a trypanosome was found.

3. The following Flagellata have been proved pathogenic to warm-blooded vertebrates when the latter have been fed, or inoculated subcutaneously or intraperitoneally with them—*Herpetomonas jaculum, H. stratiomyiae, H. pediculi, H. ctenocephali, H. culicis* and *Crithidia gerridis.* The hosts used were mice of various ages, dogs, canaries, sparrows and martins.

4. *Herpetomonas jaculum* and *Crithidia gerridis* have also been successfully fed or inoculated into cold-blooded hosts, namely, fishes (*Gasterosteus aculeatus*), frogs, toads, lizards (*Lacerta vivipara*) and grass-snakes (*Tropidonotus natrix*).

5. The disease induced may run an acute or a chronic course. In the acute cases among our vertebrates the flagellate form of the parasite was the more obvious at death. In chronic cases, non-flagellate forms of the parasite were more numerous.

6. Natural herpetomoniasis of a pigeon has been recorded by Drs. Edm. and Et. Sergent in Algeria. This affords a parallel case with the natural and induced herpetomoniasis of mice as recorded by us.

7. The flagellate stage of *Leishmania donovani* in vertebrates is now known, and that of *L. tropica* in man has been known for some time. The links completing the evidence that a Leishmania is morphologically a Herpetomonas are thus complete. We believe that leishmaniases are invertebrate-borne herpetomoniases, and that these maladies have been evolved from flagellates of invertebrates (especially herpetomonads of insects), which have been able to adapt themselves to life in vertebrates.

8. In areas where leishmaniases are endemic an examination should be made of all insects and other invertebrates likely to come into contact with men or dogs or domestic vermin like rats and mice, in order to ascertain if these invertebrates harbor herpetomonads. Preventive measures should be directed against such invertebrates, especially arthropods. Further, it is likely that members of all classes of vertebrates, and especially those members that are insectivorous, may serve as reservoirs for leishmaniases, or as they should preferably be termed, herpetomoniases. The virus may exist in such reservoirs in a very attenuated condition and so be difficult of detection. From these sources the herpetomonads may reach man by the agency of ectoparasites or flies, especially such as are sanguivorous.

ADDENDUM

As this paper—the writing of which has been greatly delayed by war work—was on the point of being despatched, our attention was drawn to an article on The Insect Vector of Uta by C. H. T. Townsend in the December number of the *Journal of Parasitology,* just received in England. The concluding paragraph of the text and more particularly of the summary of Townsend's paper were read by us with very great interest, as they confirm our conclusions regarding leishmaniases being arthropod-borne herpetomoniases. This conclusion of ours has met with considerable opposition at the hands of Wenyon, much to our

surprise, and in spite of the fact that the experiments of Laveran and Franchini, as well as the much more extended series of our own, admit of no other conclusion to our mind.

The following conclusions of ours may be compared with those of Townsend (December, 1915). Thus, in November, 1914, we stated that, "It may be expected that the various leishmaniases, occurring in different parts of the world, will prove to be insect-borne herpetomoniases." Again, in May, 1915, we wrote that: "As we have previously stated, we believe that leishmaniases are arthropod-borne herpetomoniases, and that these maladies have been evolved from flagellates of invertebrates (especially herpetonomads of insects), which have been able to adapt themselves to life in vertebrates." Further, one of us in June, 1915, wrote that: "It is inferred that the various leishmaniases are due to a herpetomonad of invertebrates which, under different conditions of environment, produces pathogenic effects in very varying degrees in different vertebrates, from zero, as in the mice described by Dutton and Todd in 1903, to high mortality as in Indian kala-azar, and probably zero again in cold-blooded hosts. It is also a flagellate which can probably live in invertebrates not already recorded as being infected. A human reservoir of leishmaniasis may occur in some places, while warm and cold-blooded vertebrates may also function as the same."

References Cited

Bayon, H. 1915. Herpetomonidae found in *Scatophaga hottentota* and *Chameleon pumilus*. Trans. Roy. Soc. S. Africa, 5: 61-63.

Chatton, E., and Blanc, G. 1914. Existence de corps leishmaniformes dans les hématoblastes d'un Gecko barbaresque, *Tarentola mauritanica*. C. R. Soc. Biol., 77: 430-433.

Dutton, J. E., and Todd, J. L. 1903. First Report of the Trypanosomiasis Expedition to the Gambia (1902). Part of Sec. VII. Flagellata in the Blood of a Mouse. Liverpool Sch. Trop. Med., Memoir, 11: 56-57.

Escomel, E. 1914. Leishmania Flagelada en el Peru. La Cronica Medica (Lima), 31: 224-227.

Fantham, H. B. 1912. *Herpetomonas pediculi,* nov. spec., parasitic in the alimentary tract of *Pediculus vestimenti,* the human body louse. Proc. Soc. Lond., B. 83: 212-227; 1 pl. 1915. Insect Flagellates and the Evolution of Disease, with Remarks on the Importance of Comparative Methods in the Study of Protozoology. Annals Trop. Med. and Parasitol., 9: 335-348.

Fantham, H. B., and Porter, A. 1914. Some Insect Flagellates Introduced Into Vertebrates. Proc. Cambr. Philos. Soc., 18: 39-50; 1 pl. 1915. Further Experimental Researches on Insect Flagellates Introduced Into Vertebrates, Proc. Cambridge Philosoph. Soc., 18: 137-148. 1915a. On the Natural Occurrence of Herpetomonads (Leptomonads) in Mice. Parasitology, 8: 128-132. 1916. Some Experimental Researches on Induced Herpetomoniasis in Birds. Annals Trop. Med. and Parasitol., 9: 543-558; 1 pl.

Franchini, G. 1913. Un nouveau protozoaire parasite de l'homme provenant du Brésil. Bull. Soc. Path. Exot., 6: 156-158.

Laveran, A., and Franchini, G. 1914. Infections de Mammifères par des flagellés d'invertébrés. Bull. Soc. Path. Exot., 7: 605-612; 4 figs.

Monge, C. 1914. La Leishmaniasis en el Peru. Espundia, Uta, Juccuya, Qcepo, Tiacc-araña. La Cronica Medica (Lima), 31:231, 251, 288, 385.

Porter, A. 1911. The Structure and Life History of *Crithidia pulicis,* n. sp., Parasitic in the Alimentary Tract of the Human Flea, *Pulex irritans.* Parasitology, 4:237-254; 1 pl.

Price, J. Dodds, and Rogers, L. 1914. The Uniform Success of Segregation Measures in Eradicating Kala-azar from Assam Tea Gardens. Its Bearing on the Probable Mode of Infection. Brit. Med. Jour., Feb. 7, pp. 285-289.

Sergent, Edm., and Et. 1907. Etudes sur les Hématozoaires d'Oiseaux. Ann. Inst. Pasteur, 21:251-280; 2 pls. (For natural Herpetomonad in pigeon, see footnote, p. 270 and Plate VII).

Wenyon, C. M. 1915. Flagellate Form of *Leishmania donovani* in the Tissues of an Experimentally Infected Dog. Jour. Trop. Med. and Hyg., 18:218-219.